SURVEILLANCE

(reference plan in progress)

HEALTH EFFECTS

Cancer

Cancer is a general term used to describe many different diseases which share a common theme of uncontrolled cell growth wth an ability to invade contiguous tissue and metastasize to distant sites. The first US Surgeon General's Report on smoking (1964) concluded that cigarette smoking causes cancer. "It has been estimated that 20% of all cancers worldwide are attributable to smoking (Parkin et al., 1999)" (IOM Report p. 367) While cigarette smoking has been linked to cancer from many different sites (e.g. bladder) lung cancer is the most notable.

Carcinogens

There are a number of chemicals in cigarette smoke that have been shown to induce the development of cancer in other settings. The information regarding the carcinogenic potential of the chenical could be derived from animal studies or epidemiological investigations. Typically, this information is then analyzed to formulate a classification with regard to the ability of the chemical (or mixture) per se to cause cancer (e.g. IARC, NTP). It is reasonable to suspect that if a chemical can cause cancer in other settings that it might cause cancer in the context of cigarette smoke. Therefore, reductions in exposure to carcinogens in smoke provide evidence leading to an anticipated reduction in morbidity or mortality. However, it certainly does not prove that this will occur.

Many chemicals which have been designated as carcinogens are found in cigarette smoke. Table 10 lists chemicals designated by either IARC, NTP, ACGIH or the EPA for which measurements have been made using Accord-JLI.

Table 1. Cigarette smoke carcinogens

(This table is not finished. It will provide a pointer to the found elsewhere in the SDS)

Chemical Class	Name	Change in Accord-JLI relative to a typical cigarette	IARC	NTP	ACGIH	EPA
Aliphatic Hydrocarbons						
	1,3-butadiene		2A			
	isoprene		2B			
Aldehydes						
	acetaldehyde		2B			
	formaldehyde		2A			
Monocyclic Aromatic Hydrocarbons						
	benzene		1			
	styrene		2B			
Polycyclic Aromatic Hydrocarbons						
	benz(a)anthracene		2A			
	benzo(b)fluoranthene		2B			
	benzo(j)fluoranthene		2B			
	benzo(k)fluoranthene		2B			
	benzo(a)pyrene		2A			
	dibenz(a,h)anthracene		2A			
	dibenz(a,e)pyrene		2B			
	dibenz(a,h)pyrene		2B			
	dibenz(a,i)pyrene		2B			
	dibenz(a,l)pyrene		2B			
	indeno(1,2,3-cd)pyrene		2B			
	5-methylchrysene		2B			
Phenols						
	catechol		2B			
Aromatic Amines						
	4-aminobiphenyl		1			

Chemical Class	Name	Change in Accord-JLI relative to a typical cigarette	IARC	NTP	ACGIH	EPA
	o-anisidine		2B			
	2-naphthylamine		1			
	o-toluidine		2B			
N-Nitrosamines						
	nitrosodibutylamine		2B			
	nitrosodiethanolamine		2B			
	nitrosodiethylamine		2A			
	nitrosodimethylamine		2A			
	nitrosodipropylamine		2B			
	nitrosomethylethylamine		2B			
	nitrosonornicotine		2B			
	nitrosopiperidine		2B			
	nitrosopyrrolidine		2B			
	NNK		2B			
Polycyclic Aza-arenes						
	dibenz(a,h)acridine		2B			
	dibenz(a,j)acridine		2B			
Aliphatic Nitrogen Compounds						
	acetamide		2B			
	acrylonitrile		2B			
	1,1-dimethylhydrazine		2B			
	2-nitropropane		2B			
	urethane		2B			
Halogen Compounds						
	vinyl chloride		1			
Metals						
	arsenic		1			
	cadmium		1			
	chromium		1			
	nickel		1			

Chemical Class	Name	Change in Accord-JLI relative to a typical cigarette	IARC	NTP	ACGIH	EPA
	lead		2B			
Inorganic Compounds						
	hydrazine		2B			

36

37 **Mode of Action**

38 There are some general modes of action which help characterize how chemicals or
39 mixtures lead to cancer. The initiation, promotion, progression model is widely
40 known.

41 Genetic damage plays a role in the initiation stage and most likely in the
42 progression stage; therefore, by inference, a reduction in exposure to smoke
43 constituents which lead to genetic damage would be reasonably anticipated to
44 decrease the likelihood of a smoker getting cancer. The yield of mutagenic
45 particulate phase smoke constituents as measured by the Salmonella reverse
46 mutation test was similarly tested under conditions in which machines were set up
47 to simulate the way people smoked in the 8-day clinical study. Reductions of > 98
48 % (tester strain TA98 with metabolic activation) to > 90 % (tester strain TA100
49 with metabolic activation) were observed comparing Accord-JLI to either Marlboro
50 Lights or Merit Ultima.

51 The kinetics of the risk of getting lung cancer in persons who stop smoking suggest
52 that cigarette smoke may act principally as a tumor promoter. Tumor promotion
53 involves sustaining epigenetic changes in the tissue which increase the probability
54 of developing a tumor. It has been suggested that the cytotoxic activity of cigarette
55 smoke might play a tumor promoting role. The yield of particulate phase and
56 gas/vapor phase smoke constituents which are cytotoxic in the neutral red dye
57 uptake assay was determined under conditions of average human smoking.
58 Reductions of >83 % (gas/vapor phase of smoke) to >85 % (particulate phase of
59 smoke) were observed when the Accord –JLI was compared to either Marlboro
60 Lights or Merit Ultima. Again, this is not proof that a reduction in cancer will be
61 observed if smokers switch to Accord-JLI but it does provide evidence that
62 exposure is going in the right direction based on current knowledge.

63

64 **Chronic Obstructive Pulmonary Disease (COPD)**

65 COPD is a disease state characterized by airflow limitations that are not fully
66 reversible. The airflow limitations are usually both progressive and associated with

an abnormal inflammatory response of the lungs to noxious particles or gases. Symptoms, functional abnormalities, and complications of COPD can all be explained on the basis of the underlying inflammation and the resulting pathology (National Heart Lung, Blood Institute 2001). *(Global Initiative for Chronic Obstructive Lung Disease (GOLD). Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. NHLBI/WHO Workshop Report. National Institutes of Health, HNLBI. Publication Number 2701, April 2001.)*

COPD is the 4th leading cause of death in the United States behind heart disease, cancer and cerebrovascular disease. (National Heart, Lung, Blood Institute 1998). In 2000, the World Health Organization (WHO) estimated 2.74 million deaths worldwide resulted from COPD (WHO 2000). According the US Surgeon General cigarette smoking is the primary cause of COPD (US Surgeon General Report, 1984).

There are three diseases which typically fall under the heading of COPD: chronic bronchitis, emphysema and small airway disease. The accepted diagnostic criteria for chronic bronchitis are cough and sputum on most days for at least 3 months for at least 2 consecutive years, without another explanation. The criteria do not include airflow obstruction. The American Thoracic Society defines emphysema as " a condition of the lung characterized by abnormal, permanent enlargement of air spaces distal to the terminal bronchiole, accompanied by destruction of their walls without obvious fibrosis" (American Thoracic Society, 1995, Am J Respir Crit Care Med 152:S77-S121, 1995). The most serious morbidity and mortality from COPD is the result of emphysema. In Small Airway Disease airways 2 mm or less in diameter become the principal sites of increased airway resistance in COPD (Hogg et al., 1968).

MKS – COMMENTS ON SDS EHCSS-JLI EDITION 1.6, FEBRUARY 27, 2003, SECTION ON COPD

INSERT AS OF LINE 747 AND DELETE CURRENT TEXT

Oxidative stress is thought to play an important role in the pathogenesis of a number of lung diseases, including chronic obstructive pulmonary disease. Cigarette smoke-induced oxidative stress has been associated with a number of constituents in mainstream cigarette smoke, as well as with oxidants secondarily formed in aqueous solution. Gas phase constituents likely to be involved are aldehydes, nitrogen oxides, and free radicals (Eiserich *et al.*, 1995). Particulate-phase cigarette smoke ("tar") contains a stable radical population which is associated with a mixture of quinone, semiquinone radical, and hydroquinone moieties held together in a polymeric matrix (Pryor *et al.*, 1983). Aqueous extracts of "tar" auto-oxidize to produce superoxide in air-saturated buffered aqueous solution (Zang *et al.*, 1995) and aqueous extracts of cigarette smoke contain large amounts of hydrogen peroxide (Pryor and Stone, 1993), which itself is the source of

hydroxyl radicals produced via the Fenton Reaction. Peroxynitrite may be formed in aqueous extracts of CS in the presence of nitric oxide and superoxide, which in turn is generated by quinone/ hydroquinone like redox systems (Müller and Gebel, 1994). Thus, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are all being formed in cigarette smoke-exposed aqueous solutions, which, as a consequence, become potent oxidants.

Oxidative damage of lipids, proteins, and DNA by cigarette smoke-derived ROS and RNS has been extensively demonstrated both *in vitro* and *in vivo*. In many cases, the initially generated reactive intermediates convert cellular constituents into second-generation reactive intermediates (e.g., acrolein, 4-hydroxynonenal) capable of inducing further damage, such as cytotoxic and genotoxic effects. When free radicals react with nonradicals (e.g., lipids), the result is a new radical, which may result in chain reactions of free radicals. Thus, relatively short-lived free radicals may propagate their damaging effects beyond the limits set by their short half-lives and limited diffusion times. In addition, cigarette smoke harbors a strong oxidative stress potential, which broadly impacts on exposed cells (Müller and Gebel, 1998). ROS/RNS activate numerous major redox sensitive signaling pathways, by directly or indirectly modulating the functions of many enzymes and transcription factors. Ultimately these signals result in changes of gene expression, which influence the ability of the cell to survive or die. This reflects the current understanding that ROS/RNS effects in cellular and molecular regulation may be mediated by oxidant-induced cellular redox imbalance. Oxidative stress arises as a consequence of the specific activation of a cascade of signaling events and may, therefore, be critical to the pro-inflammatory response to cigarette smoke.

The relationship between decreased GSH content/increased formation of GSSG to a variety of agents that impose oxidative stress is well established (Rahman and MacNee, 1996, 2000a). Exposure of cells to cigarette smoke causes rapid depletion of intracellular glutathione and this depletion parallels cell activation or toxicity (Müller and Gebel, 1998; Waldren *et al.*, 2001). The α,β-unsaturated aldehydes (acrolein, crotonaldehyde) are especially reactive electrophiles and accounted for about 50% of rapid depletion of glutathione in aqueous solutions exposed to gas phase cigarette smoke (Reddy *et al.*, 2001). The amount of oxidized glutathione (GSSG) accounted for about 25% of the decline in glutathione, possibly via RNS originating from nitrogen oxide. The decrease in intracellular glutathione content allows peroxynitrite to interfere with specific target molecules resulting in the activation of stress signal transduction and stress gene expression in cigarette smoke-treated cells *in vitro* (Müller and Gebel 1994). Cigarette smoke extract (CSE) released IL-8 from cultured human bronchial epithelial cells (Mio *et al.*, 1997). Gene expression profiling in respiratory tract tissues obtained from cigarette smoke-exposed rats revealed a pronounced activation of stress response via up-regulation of oxidative stress-related gene, many of which counteract CS-induced peroxynitrite stress (Bosio *et al.*, 2002). Acute exposure to cigarette smoke induced neutrophil infiltration into the airways in guinea pigs, which was associated with both NF-κB activation and increased IL-8 mRNA expression (Nishikawa *et al.*,1999). Prior treatment

with superoxide dismutase (SOD), an antioxidant to eliminate superoxide, inhibited neutrophil accumulation and both NF-κB activation and increased IL-8 mRNA expression. Consistent with these results, intratracheal instillation of a SOD-mimetic (AEOL 10150) provides a marked protective effect against cigarette smoke-induced inflammation and damage to the airways of rats (Smith *et al.*, 2002). The signaling pathways, transcription factors, protein, and gene targets involved in the activation of cells, as well as cellular consequences of these processes, are the subject of intense investigation. A potential mode of action of oxidant-mediated lung inflammation is depicted in Figure XX (adapted from Rahman and MacNee, 2000a). The oxidant burden in the lungs may be further enhanced in smokers by the release of ROS from macrophages and neutrophils (Rahman and MacNee, 1996, MacNee 2001).

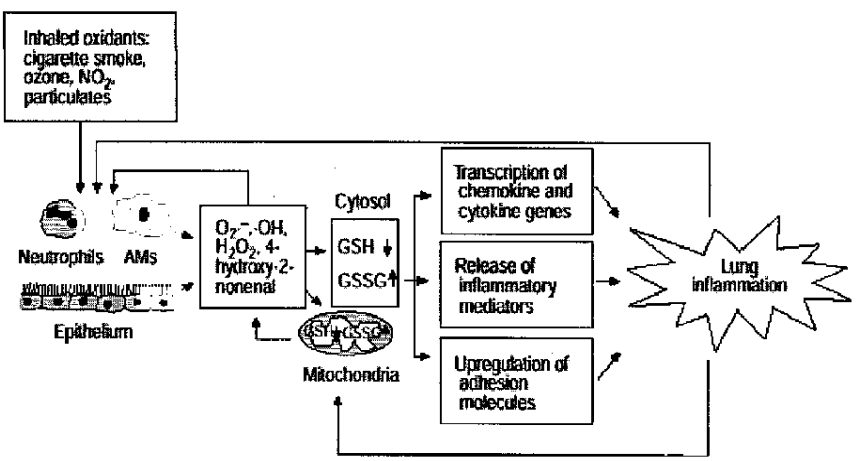

Figure XX. Adapted from Rahman 2000a

Glutathione is present in increased concentrations in the epithelial lining fluid (ELF) of the lung in chronic smokers, and is reduced in the ELF of acute smokers compared to non-smokers (Morrison *et al.*, 1999). The initial depletion is followed by a later rebound increase in GSH in epithelial cells as an adaptive response to oxidative stress, which occurs as a result of upregulation of the γ-GCS-HS gene (Rahman and MacNee, 2000b). The data presented from the smoke chemistry studies on EHCSS demonstrated a more than 90% decrease in both levels of glutathione-depleting α,β-unsaturated aldehydes (acrolein, crotonaldehyde), and nitrogen oxides. Furthermore, free radicals in the gas-phase of EHCSS are reduced by more than 90% when compared to conventional cigarettes [NOTE: The results from radical measurements conducted by the Biodynamics Institute Louisiana State University are available from Geoffrey Chan and should be included in the SDC]. Although not directly measured, it is reasonable to expect diminished glutathione depletion in subjects exposed to the smoke of EHCSS, and as a consequence, reduced burden of oxidative stress. Consistent with this expectation are the

results from the cytotoxicity experiments presented in the SDC. The cytotoxicity from the gas-phase of EHCSS-JLI smoke is markedly lower (about 75%) then that of the 1R4F when calculated on a per cigarette basis and acrolein alone was calculated to contribute about 33% of the cytotoxicity observed.

As mentioned above, aqueous extracts of cigarette tar auto-oxidize to produce hydroxyl, and superoxide radicals in air-saturated buffered aqueous solution (Zang *et al.* 1995). The aqueous phase of cigarette smoke condensate may undergo redox cycling for a considerable period of time in the epithelial lining fluid of smokers (Rahman and MacNee, 1996). Human bronchial epithelial cells exposed to cigarette smoke condensate showed increased expression of the inflammatory mediators ICAM-1, IL-1β, IL-8, and GM-GSF (Hellermann *et al.*, 2002). Cigarette smoke condensate contains free radicals, which can be directly observed by ESR. A stable radical population is associated with a mixture of quinone, semiquinone radical, and hydroquinone moieties held together in a polymeric matrix (Pryor *et al.*, 1983). A comparison of free radicals in condensate of tobacco-burning cigarettes (1R4F) and EHCSS-JLI demonstrates a reduction of radicals from the latter to below the limit of detection ($< 10^{14}$ spins/ g) [NOTE: The results from radical measurements conducted by the Biodynamics Institute Louisiana State University are available from Geoffrey Chan and should be included in the SDC]. This is consistent with the reduced (about 70%) yield in mainstream smoke phenolic compounds, including catechol and hydroquinone, of EHCSS-JLI when compared to 1R4F on an equal TPM basis. Although not directly measured, it is reasonable to expect diminished production of ROS from redox cycling in subjects exposed to the smoke of EHCSS, and as a consequence, reduced burden of oxidative stress. Consistent with this expectation are the results from the cytotoxicity experiments presented in the SDC. The cytotoxicity from the particulate-phase of EHCSS smoke is markedly lower (about 90%) then that of the 1R4F when calculated on a per cigarette basis.

Based on the expected markedly reduced oxidative stress and pro-inflammatory response imposed on exposure to cigarette smoke from EHCSS-JLI compared to a conventional cigarette, a significant reduction in lung inflammation should be expected. A 35-day inhalation study on rats was conducted to assess the relative and absolute number of neutrophils in BALF, as the main indicator of pulmonary inflammation. The results showed a >70% reduction of neutrophils in the lung lavage fluid of rats exposed to the smoke from EHCSS-JLI relative to the smoke from the 1R4F reference cigarette (Figure 23). The protease-antiprotease paradigm suggests that inflammatory cells are mainly responsible for an increase in the protease levels that degrade the connective tissue of the lung. The pathology of chronic bronchitis includes airway mucus gland hyperplasia, mucus hypersecretion, and an influx of inflammatory cells including neutrophils, macrophages, and lymphocytes. It is consequently of central importance to reduce the smoke-induced low level ongoing inflammatory process in the lower respiratory tract. As oxidants and free radicals in cigarette smoke may cause the sequestration of neutrophils from the pulmonary microcirculation as well as accumulation of macrophages in

respiratory bronchioles, and oxidants generated by inflammatory cells further stimulate the inflammatory process, a reasonable strategy is to reduce the oxidative stress associated with smoking.

Nitrogen oxides (NOx)

There is evidence to suggest that NOx may play a role in the development of COPD. Rats exposed continuously to NO (2 ppm) for six weeks were found to have significant enlargement of the airspaces and destruction of alveolar septa (Azoulay et al., 1977). Mice exposed continuously to NO (10 ppm) for 30 weeks were found to have grossly emphysematous lungs (Holt et al. 1979). However, comparable studies with mice exposured to NO_2 demonstrated only airspace enlargement (Holt et al., 1979). NO and NO_2 appear to have different mechanisms of pulmonary toxicity (Mercer et al., 1995). NO_2 principally reacts with cell membranes and is believed to cause direct cellular damage. The mechanisms of pulmonary toxicity resulting from NO exposure are unclear. However, focal degeneration of interstitial cells, interstitial matrix, and connective tissue fibers is the principal injury resulting from low level NOx exposure (rats exposed to NO or NO_2 for 9 wks at 0.5 ppm with twice daily, 1 hr spikes to 1.5 ppm) (Mercer et al., 1995). NO is more potent than NO_2 in the production of these defects.

Recent studies have utilized biomarkers such as 3-nitrotyrosine and nitrosothiols to investigate the involvement of NO-derived oxidants in many disease states (Kharitonov and Barnes 2002). Nitrotyrosine is a collective indicator of the involvement of reactive nitrogen species. High nitrosothiol values have been measured in exhaled breath condensate in smokers with COPD compared with low exhaled nitrosothiol levels in smokers without signs of COPD (Corradi et al., 2001). Furthermore, a positive correlation between nitrosothiols in exhaled breath condensate and smoking history (pack/year) was reported (Corradi et al., 2001). A significant negative correlation between FEV1 and the amount of nitrotyrosine formation has been demonstrated in patients with COPD (Ichinose et al., 2000).

The smoke from typical cigarettes contains several nitrogen oxides including NO and NO2. The predominant species is NO. The measurement technique that we have employed gives a measurement of total NOx. The results of the topography based smoke chemistry showed a >96% reduction in NOx from Accord-JLI when compared to Marlboro Lights or Merit Ultima (Figure 12).

Hydrogen Cyanide (HCN)

The waving motion of airway epithelial cell cilia help maintain airway hygiene by movement of mucus along the airway. Damage to epithelial cell cilia can result in a host inflammatory response to persistent microorganisms. If chronic, this can cause damage to the airway wall (Cole, 2001). HCN has been shown to be a cilia toxin and has been postulated to play some role in cigarette smoke-induced COPD (Kensler and Battista 1963). The results of the topography based smoke chemistry

showed a >99% reduction in HCN from Accord-JLI when compared to Marlboro Lights or Merit Ultima (Figure 12).

Lung Inflammation (redundant section to new section written by Matthias Schorp and could be deleted)

Cardiovascular Disease

The mortality rate for cardiovascular disease (CVD) in Western communities is about 49%. There are several categories of CVD: coronary heart disease/ischemic heart disease (e.g. cardiac ischemia, myocardial infarction), cerebrovascular disease (e.g. transient cerebral ischemia, stroke) and other vascular disease (e.g. aortic aneurism, peripheral vascular disease). The potential mechanisms for CVD include: atherosclerosis, hyperproliferation of the vascular wall, endothelial cell dysfunction, coagulopathy with endothelial involvement, reduced oxygen carrying capacity of blood and immune effects.

Carbon monoxide (CO) is the one constituent of cigarette smoke that stands out clearly with implications for CVD. It is the one specific chemical in cigarette smoke which is mentioned in required warnings on cigarette packaging and advertising in the US. The toxicity of CO has been studied for over a century and it has been the subject of extensive reviews (Penney, IPCS). While its principle effect is a result of binding to hemoglobin and thereby reducing the oxygen carrying capacity of blood, there are other effects. The US Surgeon General has stated: "The health effects of exposure to CO are not fully known. However, research findings in selected population groups indicate that CO acts as an added stress factor to precipitate cardiac symptomatology or ischemic episodes in individuals already compromised by coronary disease." (The Health Consequences of Smoking. 'Cardiovascular Disease'. A Report of the Surgeon General. U.S. Department of Health and Human Services 220-222 (1983))

There are many epidemiology studies which have examined the relationship between exposure to CO and CVD. The exposures were due to air pollution or the occupational environment. There are some difficulties with these studies with regard to the assessment of exposure. On issue is confounding from exposure to other substances. The other is a lack of precise exposure measurement. An examination of several of these studies (refs from Ted's CO symposium presentation) reveals a small but consistent association between CO exposure and a variety of measures of cardiovascular-related morbidity and mortality.

A large number of clinical studies have examined the effect of carbon monoxide on work and exercise capacity (refs for Roethig's CO symp presentation). Exercise is a common physiological stress used to elicit cardiovascular abnormalities not apparent at rest and to determine adequacy of cardiac function. There are numerous studies in healthy volunteers showing no cardiovascular or metabolic effects during

rest and submaximal exercise from CO exposure resulting in COHb up to 10% (HR slide 10). However in general, the acute effect of elevated COHb is a concentration dependent reduction in exercise performance, visible in healthy young subjects, but most prominent in patients with anemia, COPD or coronary artery disease. The effects are small at COHb concentrations between 2 and 4%. For example, Gliner et al. (1975) showed in healthy young males that heart rate increases significantly during 3.5 hours of submaximal exercise at COHb concentrations of up to 6.6%. Patients with coronary artery disease have earlier angina symptoms and EKG changes during exercise testing when exposed to CO resulting in 2-4% COHb (Allred et al. 1989, NEJM, slide 23). Aronow 1984 (slide 23) found that levels of 4% COHb in anemic patients decrease exercise capacity by about 20 %. Interestingly, physical work capacity in healthy smokers decreases after either smoking or CO exposure (both yielding COHb concentrations of about 9.8%). However, the decrease is greater from smoking than CO exposure alone indicating that other smoke components play a role in this effect.

Additionally, CO is produced as a part of normal cellular processes (e.g. heme oxygenase) and may play a role as a signaling molecule via its interaction with guanylyl cyclase. The shift from physiological to pathological concentrations of CO interferes with the binding of nitric oxide to guanylyl cyclase and consequently affects the concentration of free nitric oxide. Elevated free nitric oxide causes: the generation of the strong oxidant peroxynitrite, elevated heme oxygenase expression and the production of reactive iron, and apoptosis/necrosis via several pathways. Several tissues are sensitive to the toxic effects of CO based upon their physiological function to either synthesize or degrade hemoglobin, as well as the presence of high levels of heme oxygenase within the tissue.

Exposure standards have been promulgated which are designed to, in part, protect workers from adverse cardiovascular effects. An 8-hour average (TWA) limit for CO exposure (9 ppm, 3% COHb) has been set by the US EPA and the WHO based on the lowest CO level producing significant cardiac function effects (ST-segment changes, angina during exercise in subjects with coronary artery disease. (Allred et al., Environ. Health Persp., 1991, and others.) The ACGIH has set its threshold limit value (TLV) at 25ppm based on that exposure level yielding a COHb of less than 3.5%. The ACGIH has set that level in order to help prevent adverse neurobehavioral changes and reduced exercise performance due to an adverse impact on the cardiovascular system.

The design of the Accord-JLI is such that only very small amounts of CO are produced. This is indicated in the short term clinical study by the observation that the smokers of Accord-JLI had COHb levels only slightly higher than the persons who stopped smoking (Figure ??). Furthermore the COHb levels are reduced by 92% when compared with persons smoking either Merit Ultima or Marlboro Lights (Figure 7). In analysis of smoke generated by machines under a variety of puffing regimens the Accord-JLI consistently produced lower amounts of CO than typical cigarettes. For example, in smoke machines configured to mimic the topography

measured in the clinical short term study the generation of CO was reduced by >98% compared to Merit Ultima or Marlboro Lights.

In addition to CO (discussed above), other smoke constituents may play a role in the CVD related to cigarette smoking and there are a variety of thoughts about the modes of action for cigarette smoke-induced CVD. The IOM report states: "The mechanisms involved in mediating the adverse effects of cigarette smoking and of smokeless tobacco on the cardiovascular system are poorly understood, but are thought to include: induction of an adverse lipoprotein profile (Allen et al., 1994), induction of a chronic inflammatory response (Strandberg and Tilvis, 2000) including oxidative tissue injury (Morrow et al., 1995; Patrignani et al., 2000; Reilly et al., 1996; Traber et al., 1993), activation of platelets and other hemostatic variables (Benowitz et al., 1993; Ludviksdottir et al., 1999; Whiss et al., 2000), and impairment of endothelial function (Raitakari et al., 2000)".

Endothelial dysfunction has been proposed as a basic mechanism underlying the initiation of several vascular diseases (Bonetti et al, 2003). Endothelial cells line the vascular system and these cells normally function as a non-adhesive barrier between the extracellular tissues and blood-derived constituents. Endothelial cells are able to respond to numerous compounds by the elevated expression of a panel of stress and inflammatory genes (e.g., adhesive glycoproteins) and a rearrangement of the internal cytoskeleton. These chemical- or agonist-induced changes in endothelial function are designed to assist in the inflammatory response to a foreign object (e.g., virus or bacteria) by localizing adhesion of blood leukocytes to endothelial cells in the vicinity of an invading object and facilitate the migration of leukocytes into the tissue. However, prolonged changes in a quiescent non-activated endothelium in response to chemical or agonist-stimulation are recognized as a state of endothelial dysfunction and lead to continued binding of blood leukocytes, loss of barrier function and the leakage or movement of blood leukocytes, proteins and lipids into the vessel wall. This situation forms the conditions that are favorable for the migration of macrophages into the vessel wall, the deposition of lipids within sub-endothelial macrophages, the proliferation of smooth muscle cell by blood-derived growth factors, atherosclerotic plaque development, and loss of vessel function. Endothelial dysfunction is consequently linked to the initiation of atherosclerosis by the movement of leukocytes into the vasculature and these observations have led to the concept that 'atherosclerosis is an inflammatory disease' (Ross, 1999). Therefore, a reasonable strategy to reduce vascular disease in smokers, including atherosclerosis, would be to reduce those compounds in cigarette smoke that either damage endothelial cells or initiate inflammation and inflammation-mediated endothelial dysfunction. As mentioned above, the cytotoxic activity of smoke as measured by the Neutral Red Uptake assay is also reduced (Figure 19) in the Accord JLI when compared to Marlboro Lights or Merit Ultima. With regard to inflammation, the data indicating a >70% reduction of neutrophils in the lung lavage fluid of rats exposed to the smoke from EHCSS-JLI relative to the smoke for the 1R4F reference cigarette (Figure 23)

suggests that the basic inflammatory response is reduced. The decreased migration of neutrophils from the vasculature can be accounted for by one or more of the following mechanisms: a reduced activation of endothelial cells, a reduced binding of neutrophils to the activated endothelium, and/or a reduced impact on the barrier functions of the endothelium. In addition, these effects on the endothelium would be expected to extend beyond the pulmonary vasculature because cigarette smoke-derived components are known to be rapidly distributed throughout the body following their absorption. It is also relevant to mention that a key mechanism to communicate signals within the vasculature, which mediate a wide spectrum of responses (e.g., vasodilation, gene activation, cell morphology, etc.) has been identified to involve reactive oxygen species and reactive nitrogen species. The ability of these reactive molecules to elicit changes in endothelial function provides one mechanism by which continued exposure of the vasculature to oxidative stress results in endothelial dysfunction (Lum and Roebuck, 2001). In light of information, it is appropriate to note that the smoke chemistry measurements for the EHCSS demonstrate a reduced production of several compounds that affect the oxidative stress on a cell. These data include a decrease (>90%) in the levels of glutathione-depleting ~~α,β-unsaturated aldehydes (~~acrolein, ~~crotonaldehyde);~~ (Figure 11) and nitrogen oxides. ~~Free radicals in the gas-phase of EHCSS are also reduced by more than 90% when compared to conventional cigarettes.~~ Furthermore, hydrogen cyanide according to Fowles and Bates (2000) is also an initiator of cardiovascular diseases and reductions in this compound in the EHCSS would also be expected to lead to a reduction in this disease category. In summary, the reductions exhibited by the EHCSS in cytotoxicity, inflammation, and oxidative stress against cells within the lung may also extend to a reduced impact on endothelial dysfunction and endothelial-based vascular diseases.

REFERENCES

(please see email of MKS March 18, 03 for additional references on COPD; below are those new references specific for the revised CVD section)

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2. Ross, R. Atherosclerosis: An Inflammatory Disease. N.Engl. J. Med. 340, 115, 1999

3. Lum, H. and Roebuck, K.A. Oxidant Stress and Endothelial Dysfunction . Am. J. Physiol. Cell Physiol., 280, C719, 2001.

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